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## Concise Review: Organoids Are a Powerful Tool for the Study of Liver Disease and Personalized Treatment Design in Humans and Animals

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**Key Words.** Organoids • Liver • Adult stem cell culture • Applications

### ABSTRACT

**Organoids are three-dimensional culture systems in which adult stem cells and their progeny grow and represent the native physiology of the cells in vivo. Organoids have been successfully derived from several organ systems in both animal models and human patients. Organoids have been used for fundamental research, disease modeling, drug testing, and transplantation. In this review, we summarize the applications of liver-derived organoids and discuss their potential. It is likely that organoids will provide an invaluable tool to unravel disease mechanisms, design novel (personalized) treatment strategies, and generate autologous stem cells for gene editing and transplantation purposes.**

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### SIGNIFICANCE

Organoids derived from the liver have hepatocellular differentiation potential and can be an unlimited source for hepatocytes for application in in vitro toxicology testing and for transplantation purposes as an alternative to orthotopic liver transplantation. The in vitro representation of the native physiology and epigenetic background of the adult liver stem cells makes the organoid technology an excellent tool to study and model liver diseases, for drug screening, and for the design of personalized treatments. This review summarizes the applications of liver organoids and discusses their potential in the study and modeling of liver diseases, and in the development and testing of novel drugs.

### INTRODUCTION

There has been increased interest in adult hepatic stem/progenitor cells (HPCs) because they have the potential to provide an unlimited source of hepatocytes. Cell therapy may be an important alternative to orthotopic liver transplantation [1–3]. In addition, human hepatocytes are also the gold standard for in vitro toxicology tests. Unfortunately, availability of mature hepatocytes is limited because of their low replicative potential in vitro [3], rapid dedifferentiation during culture [4], and loss of viability and function following cryopreservation [5]. Efficient generation of functional hepatocytes or hepatocyte-like cells from HPCs with unlimited expansion capacity in vitro would enable effective stem cell-based therapies and toxicology studies. In addition, it would boost the research field by providing a valuable cell source for fundamental research, disease modeling, drug screening, and personalized medicine.

HPCs are considered a good eligible source for hepatocytes and hepatocyte-like cells, compared with embryonic stem cells (ESCs) or induced

pluripotent stem cells (iPSCs): HPC differentiation is more efficient and less time consuming [6], HPCs have lower risk for spontaneous mutations, chromosomal rearrangements, and teratoma formation [7], and they are less of a concern ethically. To harness the full potential of HPCs, a robust method for the isolation and culture of these cells from patients with liver disease is essential. This review discusses the potential of HPCs in the form of liver organoids and their advantages and disadvantages. In particular, we focus on the recently developed liver organoid technology, and do not include three-dimensional (3D) cocultures, which are also often defined as liver organoids elsewhere in the literature.

### CULTURES OF HEPATIC STEM/PROGENITOR CELLS

There have been many attempts at isolating HPCs for long-term culture. Traditionally, two-dimensional (2D) culture of HPCs is relatively easy and economical; however, long-term culture

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without loss of stem cell characteristics is difficult [8], and chromosome alterations often occur with higher passages [9]. Moreover, cells grown in 2D conditions fail to recapitulate in vivo cell polarization and cellular interactions, and poorly maintain in vivo cell characteristics required for proper function. Finally, tissue-specific architecture, mechanical and biochemical cues, and cell-cell communication are absent under 2D conditions [10].

The development of 3D liver cell cultures partly circumvents these issues, allowing hepatocyte polarization and interactions between cells and their microenvironment [4]. Recently, a 3D culture system of adult HPCs was developed with genetically stable, long-term (>1 year) expansion. These cultures were derived from fluorescence-activated cell-sorted Lgr5<sup>+</sup> (injured mouse liver) and EpCAM<sup>+</sup> (normal human liver) ductal cells, and from biliary duct fragments from normal and/or diseased murine and human livers [11, 12]. The cultures were grown in laminin-rich Matrigel. Many characteristics of the cells' original epithelial architecture were maintained, and the organoids were differentiated in vitro toward hepatocytes and cholangiocytes. Upon transplantation into injured mouse livers, the organoids gave rise to morphologically and functionally mature hepatocytes [11, 12].

A great benefit of organoids is that they can be isolated from patients by using less invasive Tru-cut needle biopsies or fine-needle aspiration. Biopsied tissues are enzymatically digested to retrieve biliary duct fragments that grow into organoids, whereas fine-needle aspirates provide cells for direct culture, resulting in organoid growth. We observed that tissues kept in a cold medium for 2 days, frozen in dimethylsulfoxide-based freezing medium or immediately snap-frozen in liquid nitrogen, were suitable for liver organoid isolation. Moreover, the organoids themselves can be cryopreserved and recovered, which is of critical importance for cell banking [11]. Together, this means that collecting cells and subsequent culture of organoids can be from various sources and are not limited to highly invasive liver surgery. This recent development of 3D liver cell culture is therefore very attractive for future translational applications.

## APPLICATIONS OF ORGANOID

The potential applications for and pros and cons of liver organoids are summarized in Figure 1 and Table 1, respectively.

### Organoids for Fundamental Research

Organoids are currently considered one of the better platforms for studying developmental questions regarding cell differentiation and maturation. In particular, we can direct the differentiation of liver organoids toward hepatocyte-like cells by Notch inhibition and/or Wnt signaling [13]. Moreover, transcriptional factors and regulatory pathways involved in organ specification and late cell-fate determination can be investigated during organoid development to gain a better understanding of disease development and progression.

### Organoids for Disease Modeling

Genetic modification of HPCs to create disease-specific models in vitro is of specific interest for rare diseases like Wilson disease, especially when acquisition of patient material is challenging. For example, human organoids derived from  $\alpha$ 1-antitrypsin deficiency and patients with Alagille syndrome reflect the in vivo pathologies of these diseases; apparently, these genetic defects did

not influence organoid proliferation [12]. This in vitro reflection of in vivo phenotype provides opportunities to investigate disease mechanisms and develop new treatment strategies and, at the same time, reduce experimental animal use. To date, liver organoids are only used to model genetic liver diseases. In this article, we discuss the potential use of liver organoids in other research areas.

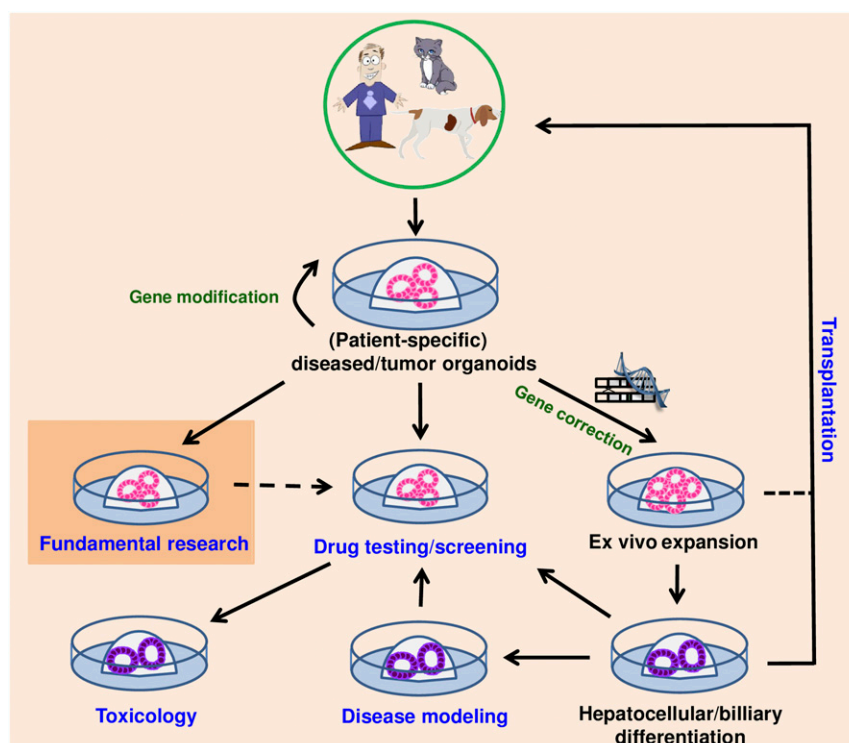
### Liver Organoids to Study Viral Hepatitis

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses that affect more than 350 million people worldwide. The ability to propagate these viruses in vitro is crucial to obtain a detailed understanding of virus-host cell interaction [14]. The lack of suitable cell cultures for HBV and HCV has hampered the analysis of the viral life cycle and drug discovery. To date, there is no in vitro experimental system for viruses that supports the entire viral life cycle, sustains high viral entry and replication, and permits patient-derived viral isolates [14, 15]. Primary human hepatocyte cultures represent the most physiologically relevant system and are highly susceptible to HBV and HCV infection [16, 17]. However, their limited availability, short life span in culture, and low viral replication in hepatocytes because of hepatic interferon- $\gamma$  production have impeded the use of this model [18, 19]. The human hepatoma cell line Huh7.5 is currently the most widely used cell system because these cells can sustain high HCV titers. A significant drawback of Huh7.5 cells is lack of polarity and, therefore, nonproductive infection of patient-derived isolates [15]. A recent report described the first human hepatoma cell line, HLCZ01, that permits direct HBV and HCV infection by patient sera [18]. In addition to hepatocellular cell lines and primary hepatocytes, stem cell-derived hepatocyte-like cells have gained interest. Several studies have demonstrated that iPSC- and/or ESC-derived hepatocyte-like cells can be infected with HCV [15].

Despite availability of these in vitro models, there are still some issues that cannot be addressed simultaneously with the current models (e.g., host-virus interaction [20], the budding-off process of infectious particles). HPCs have been shown susceptible to HCV infection and, unlike pluripotent stem cells and definitive endoderm, are able to bud-off infectious particles [19, 21]. Based on these arguments, organoids could be an excellent model for the study of viral hepatitis. It is expected that hepatocyte-like cells differentiated from patient-derived organoids could overcome the current limitations for several reasons. First, organoids retain their innate immune response. Second, the 3D structure of differentiated liver organoids allows for correct cell polarization, facilitating HBV and HCV cell entry and cell-to-cell transmission. Third, the possibility of performing genetic modification in long-term organoid cultures holds great promise for generating virus-resistant hepatocyte-like cells for potential life-saving therapy. Thus, organoids may provide an excellent culture system to study and to model viral hepatitis.

### Liver Organoids to Study Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) has a 91% prevalence in obese patients, with an astonishingly high incidence in children. NAFLD is a multifaceted and complicated disease characterized by steatosis. Patients with NAFLD may develop nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and, eventually, cancer. Generally, NAFLD is caused by interactions between



**Figure 1.** Application for liver organoids in regenerative medicine and cancer. The scheme represents the potential of liver organoids for fundamental research and translation to the clinic. Organoids are isolated and cultured from human and animal patients. Organoids can be genetically modified for cancer research and rare diseases. Gene correction can be performed ex vivo in patient-specific organoids before autologous transplantation. Upon differentiation, organoids may be used for multiple purposes, including disease modeling, drug testing, toxicology, and transplantation, that are essential for development of personalized and precision therapies.

environmental and genetic factors; however, the precise pathogenesis and transition to NASH are not clearly understood [22–24]. This warrants the development of experimental models that mimic the human condition. Previous studies using primary hepatocyte cultures, immortalized cell lines, cocultures of hepatocytes with adipocytes, and precision-cut slices of perfused liver have confirmed several pathways involved in NAFLD-to-NASH progression, both in vitro (human) and in vivo (animal models) [23]. Unfortunately, animal experimental models, although advantageous in defining cause-and-effect relationships, do not fully recapitulate human NAFLD/NASH with respect to the degree of hepatocellular damage and metabolic alterations [23]. Pure hepatocyte cultures enable investigation into genetic stability and/or fat metabolism during NAFLD/NASH transition. Fibrosis, on the other hand, is a complex interplay between various cell types and obviously requires more complex coculture systems [25]. In this respect, liver organoid-derived hepatocyte-like cells may be advantageous. Because the genetic signature of a patient is maintained and cultures are stable in the long term, the influence of genetic background and hepatocellular pathways of NAFLD/NASH becomes testable. In addition, epigenetic alterations important in the development and progression of NAFLD [24] can be specifically studied in patient-derived organoids. Elucidation of epigenetic factors may identify early diagnostic biomarkers and therapeutic strategies for NAFLD.

A more comprehensive approach to investigating the intercellular mechanisms of NAFLD and progression to NASH includes coculture of organoids with other cell types (e.g., stellate cells, Kupffer cells, other inflammatory cells). In addition, tissue

engineering and 3D printing technologies provide further opportunities to mimic the complex liver in vitro. Finally, organoid technology has been established for several species, including human, mouse, and dog [13]; most recently, we have succeeded in culturing organoids from various cat livers. Taken together, we are now in a position where technologies can complement relevant animal models, and parallel investigations into various species with different disease susceptibilities only strengthen our translational approaches.

### Organoids for Personalized Medicine

The clinical potential of patient-derived organoids has been reported for patients suffering from cystic fibrosis, which is caused by a mutation in the *CFTR* gene. *CFTR* function was efficiently restored in organoids (intestinal, colon, and pulmonary) derived from these patients, either by chemical compounds or by the CRISPR-Cas9 genome editing strategy [26–28]. This allowed pre-clinical ex vivo testing of new *CFTR*-targeted drugs to customize treatments for individual patients or to target specific *CFTR* mutations (precision therapy) [29, 30]. It is conceivable that this approach may be similarly effective for liver organoids.

### Liver Organoids and Hepatocellular Carcinoma

Human hepatocellular carcinoma (HCC) is associated with a high prevalence of chromosome copy-number alterations and translocations, as well as somatic mutations. The genetic heterogeneity, even within the same pathological tumor classification, affects clinical features, clinical care, and prognosis of the individual

**Table 1.** Pros and cons of liver organoid technology

| Application                         | Pros   | Cons   |
|-------------------------------------|--|--|
| Fundamental research                | Studies in <ul style="list-style-type: none"> <li>• Stem cell biology</li> <li>• Cell-cell interaction</li> <li>• Cell-ECM interaction studies (in coculture)</li> </ul> | Studies in cell biology of fully mature hepatocytes or cholangiocytes, because of the low differentiation potential.   |
| Disease modeling                    | <ul style="list-style-type: none"> <li>• Cell banking possibility</li> <li>• Patient-derived cells with low invasive techniques</li> </ul>                               | Disease models with fully mature epithelial cells will require improved organoid differentiation.  |
| Precision and personalized medicine | <ul style="list-style-type: none"> <li>• Patient-specific cell culture systems</li> <li>• Potential for genome editing</li> </ul>  | Selective procedures for corrected clones are needed.  |
| Drug screening and toxicology       | <ul style="list-style-type: none"> <li>• Suitable for long-term testing</li> </ul>   | Mature hepatocytes require improved differentiation methods.   |
| Transplantation                     | <ul style="list-style-type: none"> <li>• No risk for teratoma</li> <li>• Potential for gene correction and autologous transplantation</li> </ul>                         | <ul style="list-style-type: none"> <li>• Low differentiation potential</li> <li>• New protein expression upon gene correction requiring immunosuppression</li> </ul> |

Abbreviation: ECM, extracellular matrix.

patient [31]. It has been demonstrated that pancreatic organoids generated from neoplastic murine and human pancreas tissues [32], upon transplantation, recapitulate the pathophysiology of pancreatic tumor development. Transcriptional and proteomic analyses identified expression of the same genes and pathways in these organoids and their original human tissues [32]. Similar results were obtained in organoids derived from colorectal carcinoma [33]. In our laboratory, we were able to culture organoids from liver tumors and normal tissues simultaneously from the same patient, providing the opportunity to perform deep sequencing analysis to identify mutations and altered signaling pathways in individual tumors. We anticipate that testing specific anticancer drugs in patient-derived tumor organoids will consequently provide predictive data on patient-specific treatment efficacy.

### Organoids for Transplantation

Liver organoids are attractive for transplantation, because of their potential to be substitutes for primary hepatocytes, which, themselves, demonstrate excellent posttransplant repopulation capacity. Liver organoids expand rapidly; allow autologous transplantation, thus avoiding immunoreaction; and are genetically stable. Upon transplantation in mice, neither dysplastic nor anaplastic growth was found in any of the organoid-transplanted recipient mice [11, 12, 34]. This contrasts with iPSCs, which are known to have an unpredictable degree of differentiation and a higher risk for teratoma formation [7, 35]. These characteristics strongly indicate that organoid transplantation may be a safer counterpart to iPSC-based strategies. To fully rescue metabolic or genetic defects, adequate engraftment efficiency of transplanted cells is necessary. Currently, only 1% engraftment of organoids has been reported [11], whereas at least 10% engraftment is required to restore enzyme or protein deficiency [3, 36]. Strategies such as priming the recipient liver may improve organoid engraftment. A tremendous ethical and logistical advantage of liver organoids is that they can be grown from liver biopsy specimens taken from living donors or patients by relatively minimally invasive techniques. Because gene manipulation can be

performed in patient-derived organoids ex vivo, the corrected organoids can be selectively expanded to reach desired numbers for autologous transplantation.

## ORGANOIDS IN ANIMALS

### Canine and Feline Liver Progenitor Cultures

There is much need for new regenerative treatment strategies for veterinary patients. This is especially true for companion animals, where the standard of care approaches the level of human clinical care. Many liver diseases in dogs and cats develop in the same manner as observed in human patients [37, 38]. It is, therefore, conceivable that organoid generation from these species may accelerate the translational benefit in human medicine, with the added advantage of fewer ethical and administrative obstacles.

Several canine adult HPC culture systems have been available since the early 1990s—for example, the canine bile duct epithelial cell line, established from gall bladder epithelial cells [39]; a hepatocellular tumor cell line, which was derived from a clinical/spontaneous HCC in a dog [40]; and a primary nonparenchymal cell fraction enriched with small hepatocytes and small epithelial cells from normal, mature dog livers [38]. Despite this, a pure HPC culture system with long-term, genetically stable culture potential was not achieved until recently. To our knowledge, there are still no HPC culture systems available for felines. Our recently established canine and feline liver organoids may provide an alternative for the development of transplantation therapy, investigation of disease mechanisms, and development of personalized treatment strategies.

### Liver Organoids to Study Canine and Feline Liver Diseases and Cancers

Hepatitis is one of the most common liver diseases in dogs. Because the etiology is most often unknown, therapeutic strategies rely on symptoms and histopathological descriptions [41]. Canine hepatitis and human viral hepatitis share many characteristics, including similar molecular pathways, histopathological lesions, clinical signs, and disease progression [37]. It is generally thought,



therefore, that canine hepatitis, like human hepatitis, is viral in origin; however, its identity and nature remain elusive [41]. Canine liver organoids could provide us with a tool for testing hepatocellular infectivity and viral propagation to develop targeted treatment strategies.

The most common form of liver disease in cats is hepatic lipidoses, for which obese animals are at higher risk [42] and which resembles human NAFLD. Because cats are considered an excellent model for the study of metabolic diseases in humans [43], feline liver organoids enable us to better understand individual differences in susceptibility and to identify cellular and molecular mechanisms of feline and human hepatic lipid-storage diseases. Another common liver disease in cats is lymphocytic cholangitis, a classification of hepatobiliary disease [44]. This disease has similar histological features as primary sclerosing cholangitis and primary biliary cirrhosis (PBC) in humans; however, the etiology and pathogenesis of these diseases are not clearly defined [45]. Liver organoids derived from biliary duct fragments of feline patients could be an effective tool for in vitro analysis of primary biliary function and morphology [13], as well as drug screening. Moreover, organoids with a bipotential differentiation capacity may be able to renew at least some of the defective biliary trees in patients, providing an alternative for liver transplantation. An established model of primary sclerosing cholangitis/PBC for feline cholangitis could lead to novel combination therapies, which, in turn, may facilitate new drug development for human clinical practice.

Finally, cancer may soon become the leading cause of death in companion animals, as a result of increased life span. It has been observed that the same cancer types often develop in both humans and companion animals [46]. The development of novel treatment strategies and personalized treatments for tumors in pets, using organoid technology, will, therefore, mutually benefit the human and veterinary fields.

### CHALLENGES OF USING LIVER ORGANIDS

Liver organoids are available for several species and open new avenues for liver therapeutics [11–13]. Rodents remain important models for fundamental studies; dogs and cats serve to accelerate preclinical translational models at low cost; and human organoids are of incredible value for the analysis of patient-specific disease mechanisms and treatment efficacy. Because of similarities in disease development, canine HPC transplantation may provide a bridge from fundamental findings to the human clinic.

Although full hepatic differentiation is not critical for transplantation purposes [11, 12], it is essential to improve the in vitro efficiency of hepatic differentiation for disease modeling and preclinical pharmacotoxicological studies. This may be achieved

through adaptation of media composition and culture components. For example, recapitulation of the in vivo extracellular matrix (ECM) remains challenging. It has been demonstrated that the use of decellularized liver ECM improves primary hepatocyte culture function [47], and we suggest this could have similar effects on organoid-derived hepatocyte-like cells. Further development of coculture organoid systems, along with improving spatial architecture (e.g., 3D printing), may enable more efficient hepatic differentiation.

Generating effective 3D liver organoids, however, is not without its own challenges. First, it is laborious and expensive. Second, organoids are cultured in a matrix environment, requiring alternative approaches to sample handling, manipulation, and functional assays. Finally, to analyze intact 3D structures, novel imaging and quantitative analysis techniques must be implemented. Fortunately, these techniques are evolving concurrently with organoid technology, and published transfection, transduction [48], high-throughput screening [33], and 3D imaging and analysis techniques [13] demonstrate that this convergence of tools will facilitate the powerful application of organoids for regenerative strategies.

### CONCLUSION

To date, 3D organoid technology is the best available representation of the in vivo physiology of native epithelial liver cells. Organoid cultures have greatly enhanced our underlying knowledge of disease development and progression, have demonstrated faithful recapitulation of disease pathways in vivo, and are currently available from a variety of species. These elements, together with emerging technologies (such as 3D printing and imaging techniques), and the abundance of opportunities in research, drug discovery, and patient-specific applications, indicate that organoids may significantly contribute to the changing landscape of how diseases are studied and medicine developed.

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### AUTHOR CONTRIBUTIONS

S.N.: manuscript writing; A.d.B. and J.R.: conception and design, final approval of manuscript; L.C.P.: manuscript writing, final approval of manuscript; B.A.S.: conception and design, manuscript writing, final approval of manuscript.

### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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